

WHAT IS CLAIMED IS:

- 1 1. A protein having ~~an~~ amino acid sequence that is substantially similar to that of
2 SEQ ID NO:3.
- 1 2. The protein of Claim 1 having the amino acid sequence of SEQ ID NO:3.
- 1 3. The protein of Claim 1 purified to exhibit a single protein band on 7% SDS-
2 PAGE.
- 1 4. The protein of Claim 1 comprising a converted cysteine.
- 1 5. The protein of Claim 4 wherein the converted cysteine is an alkylated cysteine.
- 1 6. The protein of Claim 4 wherein the converted cysteine is a cysteine substituted
2 with an alternative polar neutral amino acid.
- 1 7. The protein of Claim 6 wherein the alternative polar neutral amino acid is selected
2 from the group consisting of a glycine, a serine, and a threonine.
- 1 8. The protein of Claim 4 comprising three converted cysteines, wherein the
2 converted cysteines are Cysteine 155, Cysteine 440, and Cysteine 492.
- 1 9. The protein of Claim 8 wherein the three converted cysteines are alkylated
2 cysteines.
- 1 10. The protein of Claim 9 comprising a phosphorylated tyrosine at tyrosine 701.
- 1 11. The protein of Claim 10 purified to exhibit a single protein band on 7% SDS-
2 PAGE.
- 1 12. A purified N-terminal peptide fragment of a Stat protein having an amino acid
2 sequence substantially similar to SEQ ID NO:4.

1 13. The purified N-terminal peptide fragment of Claim 12 having an amino acid
2 sequence of SEQ ID NO:4.

1 14. A nucleic acid that comprises a nucleotide sequence that codes for the expression
2 of a truncated Stat protein having an amino acid sequence that is substantially similar to
3 that of SEQ ID NO:3.

1 15. The nucleic acid of Claim 14 wherein the nucleotide sequence is SEQ ID NO:5.

1 16. A nucleic acid comprising a nucleotide sequence encoding the N-terminal peptide
2 fragment of a Stat protein having an amino acid sequence substantially similar to SEQ ID
3 NO:4.

1 17. The nucleic acid of Claim 16 wherein the nucleotide sequence is SEQ ID NO:6.

1 18. A method of separating the phosphorylated form of a protein from the
2 nonphosphorylated form comprising:

3 (a) placing a mixture of the phosphorylated form of a protein and the
4 nonphosphorylated form of the protein onto heparin agarose; wherein the phosphorylated
5 form of the protein and the nonphosphorylated form of the protein bind to heparin
6 agarose;

7 (b) eluting the phosphorylated form of the protein and the nonphosphorylated
8 form of the protein from the heparin agarose as a function of salt concentration;
9 wherein the nonphosphorylated form of the protein elutes prior to the
10 phosphorylated form of the protein; and

11 wherein the protein is selected from the group consisting of a Stat protein and a
12 truncated Stat protein.

1 19. The method of Claim 18 wherein eluting the heparin agarose as a function of salt
2 concentration is performed with a salt gradient.

1 20. The method of Claim 18 wherein the protein is a Stat protein having an amino
2 acid sequence selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:2.

1 21. The method of Claim 18 wherein the protein is a truncated Stat protein having an
2 amino acid sequence substantially similar to SEQ ID NO:3.

1 22. The method of Claim 18 wherein eluting the heparin agarose as a function of salt
2 concentration is performed stepwise with an approximately 0.15 M monovalent salt elution
3 step, followed by an approximately 0.4 M monovalent salt elution step; and wherein the
4 unphosphorylated form of the protein elutes with the first step and the phosphorylated
5 form of the protein elutes with the second step.

1 23. The method of Claim 22 wherein the protein has a converted cysteine.

1 24. The method of Claim 23 wherein the protein is a Stat protein having an amino
2 acid sequence selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:2.

1 25. The method of Claim 23 wherein the protein is a truncated Stat protein having an
2 amino acid sequence that is substantially similar to SEQ ID NO:3.

1 26. A method of preparing a purified alkylated Stat protein comprising:

2 (a) placing an expression vector containing a nucleic acid encoding a Stat
3 protein into a compatible host cell, wherein the Stat protein is expressed;

4 (b) growing the compatible host cell;

5 (c) releasing the expressed Stat protein from the host cell;

6 (d) alkylating a cysteine of the expressed Stat protein, wherein the cysteine is
7 involved in intersubunit aggregation, and wherein an alkylated Stat protein is formed; and

8 (e) isolating the alkylated Stat protein; wherein said isolating yields a purified
9 alkylated Stat protein.

1 27. The method of Claim 26 wherein the Stat protein has an amino acid sequence
2 selected from the group consisting of SEQ ID NO:1, and SEQ ID NO:2.

1 28. The method of Claim 27 further comprising the step of phosphorylating the
2 alkylated Stat protein.

1 29. The method of Claim 27 wherein said alkylating is performed by incubating the

2 Stat protein with N-ethyl maleimide.

- 1 30. A method of preparing a purified alkylated truncated Stat protein comprising:
 2 (a) placing an expression vector containing a nucleic acid encoding a truncated
 3 Stat protein into a compatible host cell, wherein the truncated Stat protein is expressed;
 4 (b) growing the compatible host cell;
 5 (c) releasing the expressed truncated Stat protein from the host cell;
 6 (d) alkylating a cysteine of the expressed truncated Stat protein, wherein the
 7 cysteine is involved in intersubunit aggregation, and wherein an alkylated truncated Stat
 8 protein is formed; and
 9 (e) isolating the alkylated truncated Stat protein; wherein said isolating yields
 10 a purified alkylated truncated Stat protein;

11 wherein the truncated Stat protein has an N-terminal sequence that is substantially
 12 similar to the N-terminus of the corresponding Stat protein following the cleavage of the
 13 proteolytic sensitive N-terminal domain from the corresponding Stat protein; and
 14 wherein the carboxyl terminus of the truncated Stat protein extends at least to the
 15 phosphorylatable tyrosine required for homodimerization.

- 1 31. The method of Claim 30 wherein about 40 to 50 mg of purified alkylated
 2 truncated Stat protein can be obtained from 6 liters of starting culture.

- 1 32. The method of Claim 30 further comprising the step of phosphorylating the
 2 alkylated truncated Stat protein.

- 1 33. The method of Claim 32 wherein the truncated Stat protein has an amino acid
 2 sequence that is substantially similar to SEQ ID NO:3.

- 1 34. A method of preparing a purified substituted Stat protein comprising:
 2 (a) placing an expression vector into a compatible host cell, wherein the
 3 expression vector contains a nucleic acid encoding a substituted Stat protein that has an
 4 alternative polar neutral amino acid substituted for a cysteine of the Stat protein, wherein
 5 the cysteine is involved in intersubunit aggregation, and wherein the substituted Stat
 6 protein is expressed;
 7 (b) growing the compatible host cell;

- 8 (c) releasing the expressed substituted Stat protein from the host cell; and
9 (d) isolating the substituted Stat protein; wherein said isolating yields the
10 purified substituted Stat protein.

1 35. The method of Claim 34 wherein the Stat protein has an amino acid sequence
2 selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:2.

1 36. The method of Claim 34 further comprising the step of phosphorylating the
2 substituted Stat protein.

1 37. The method of Claim 34 wherein the alternative polar neutral amino acid is
2 selected from the group consisting of a glycine, a serine, and a threonine.

1 38. A method of preparing a purified substituted truncated Stat protein comprising:

2 (a) placing an expression vector into a compatible host cell, wherein the
3 expression vector contains a nucleic acid encoding a substituted truncated Stat protein that
4 has an alternative polar neutral amino acid substituted for a cysteine of the truncated Stat
5 protein, wherein the cysteine is involved in intersubunit aggregation, and wherein the
6 substituted truncated Stat protein is expressed;

7 (b) growing the compatible host cell;

8 (c) releasing the expressed substituted truncated Stat protein from the host
9 cell; and

10 (d) isolating the substituted truncated Stat protein; wherein said isolating
11 yields the purified substituted Stat protein;

12 wherein the truncated Stat protein has an N-terminal sequence that is substantially
13 similar to the N-terminus of the corresponding Stat protein following the cleavage of the
14 proteolytic sensitive N-terminal domain from the corresponding Stat protein; and

15 wherein the carboxyl terminus of the truncated Stat protein extends at least to the
16 phosphorylatable tyrosine required for dimerization.

1 39. The method of Claim 38 wherein about 40 to 50 mg of purified substituted Stat
2 protein can be obtained from 6 liters of starting culture.

1 40. The method of Claim 38 further comprising the step of phosphorylating the

2 substituted truncated Stat protein.

1 41. The method of Claim 40 wherein the truncated Stat protein has an amino acid
2 sequence that is substantially similar to SEQ ID NO:3.

1 42. A method for identifying a drug that enhances or diminishes the ability of STAT
2 protein dimers to induce the expression of a gene operably under the control of a promoter
3 containing at least two adjacent weak binding sites for STAT protein dimers comprising:

4 (a) measuring the level of expression of a first reporter gene and a second
5 reporter gene contained by a host cell in the presence and absence of a prospective drug;
6 wherein the first reporter gene is operably linked to a first promoter containing at least
7 two adjacent weak binding sites for STAT protein dimers, and the second reporter gene is
8 operably linked to a second promoter comprising at least one strong binding site for a
9 STAT protein dimer; wherein the binding of STAT protein dimers to the two adjacent
10 weak binding sites induces the expression of the first reporter gene, and wherein the
11 binding of the STAT protein dimer to the strong binding site induces the expression of the
12 second reporter gene; and wherein the host cell contains STAT protein dimers;

13 (b) comparing the level of expression of the first reporter gene with that of the
14 second reporter gene in the presence and absence of the prospective drug, wherein when
15 the presence of the prospective drug results in an increase in the level of expression of the
16 first reporter gene but not that of the second reporter gene, the prospective drug is
17 identified as a drug that enhances the ability of STAT protein dimers to induce the
18 expression of a gene operably under the control of a promoter containing at least two
19 adjacent weak binding sites for STAT protein dimers; and when the presence of a
20 prospective drug results in a decrease in the level of expression of the first reporter gene
21 but not that of the second reporter gene the prospective drug is identified as a drug that
22 inhibits the ability of STAT protein dimers to induce the expression of a gene operably
23 under the control of a promoter containing at least two adjacent weak binding sites for
24 STAT protein dimers.

1 43. The method of Claim 42 wherein the host cell is a mammalian cell.

1 44. The method of Claim 42 wherein the first reporter gene is contained by a first host
2 cell, and the second reporter gene is contained by a second host cell; and wherein the first

3 host cell and second host cell both contain STAT protein dimers.

1 45. The method of Claim 42 wherein the weak STAT binding sites are selected from
2 the group consisting of sites present in the regulatory regions of the MIG gene, the c-fos
3 gene and the interferon- γ gene.

1 46. A method for identifying a drug that modulates the ability of adjacent STAT
2 protein dimers to interact and bind to adjacent DNA binding sites comprising:
3 a) measuring the level of expression of a reporter gene in a first host cell in
4 the presence and absence of a test compound, wherein the first host cell contains a
5 reporter gene operably linked to a promoter comprising at least two adjacent weak binding
6 sites for the STAT protein dimer, such that binding of the dimers to the promoter causes
7 expression of the reporter gene;

8 b) measuring the level of expression of a reporter gene in a second host cell
9 in the presence and absence of the test compound, wherein the second host cell contains a
10 reporter gene operably linked to a second promoter comprising at least one strong binding
11 site for the STAT protein dimer, such that binding of the dimer to the promoter causes
12 expression of the reporter gene; and

13 c) comparing the level of expression of the reporter gene in the first host cell
14 in the presence and absence of the test compound with the level of expression of the
15 reporter gene in the second host cell in the presence and absence of the test compound,
16 wherein a test compound which causes an increase in the level of expression of the
17 reporter gene in said first host cell but not in said second host cell is identified as a drug
18 that enhances the interaction between adjacent STAT protein dimers, and a test compound
19 which causes a decrease in the level of expression of the reporter gene in the first host cell
20 but not in the second host cell is identified as a drug that inhibits the interaction between
21 adjacent activated STAT dimers.

1 47. The method of Claim 46 wherein the first host cell and second host cell are
2 mammalian cells.

1 48. The method of Claim 46 wherein the weak STAT binding sites are selected from
2 the group consisting of sites present in the regulatory regions of the MIG gene, the c-fos
3 gene and the interferon- γ gene.

1 49. A method for identifying a drug that modulates the ability of adjacent STAT
 2 protein dimers to interact and bind to adjacent DNA binding sites comprising:
 3 a) measuring the binding affinity of the STAT protein, or a fragment thereof
 4 that comprises the N-terminal domain, to a nucleic acid comprising 2 adjacent weak STAT
 5 DNA binding sites in the presence and absence of a test compound:
 6 b) measuring the binding affinity of the STAT protein, or the fragment, to a
 7 nucleic acid comprising a single strong STAT binding site in the presence and absence of
 8 the test compound; and
 9 c) comparing the binding affinity measured in step (a) in the presence and
 10 absence of the test compound with the binding affinity measured in step (b) in the
 11 presence and absence of the test compound, wherein a test compound which causes an
 12 increase in the binding affinity measured in step (a) but not in the binding affinity
 13 measured in step (b) is identified as a drug that enhances the interaction between adjacent
 14 activated STAT dimers, and a test compound which causes a decrease in the binding
 15 affinity measured in step (a) but not in the binding affinity measured in step (b) is
 16 identified as a drug that inhibits the interaction between adjacent activated STAT dimers.

1 50. A method for identifying a drug that modulates the ability of adjacent STAT
 2 protein dimers to interact and bind to adjacent DNA binding sites comprising:
 3 a) measuring the binding affinity of the STAT protein, or a fragment thereof
 4 comprising the N-terminal domain, to a nucleic acid comprising 2 adjacent weak STAT
 5 DNA binding sites in the presence and absence of a test compound;
 6 b) measuring the binding affinity of a truncated form of the STAT protein
 7 lacking the N-terminal domain with the nucleic acid in the presence and absence of the
 8 test compound; and
 9 c) comparing the binding affinity measured in step (a) in the presence and
 10 absence of the test compound with the binding affinity measured in step (b) in the
 11 presence and absence of the test compound, wherein a test compound which causes an
 12 increase in the binding affinity measured in step (a) but not in the binding affinity
 13 measured in step (b) is identified as a drug that enhances the interaction between adjacent
 14 activated STAT dimers, and a test compound which causes a decrease in the binding
 15 affinity measured in step (a) but not in the binding affinity measured in step (b) is
 16 identified as a drug that inhibits the interaction between adjacent activated STAT dimers.

1 51. A method for identifying a drug that modulates the ability of adjacent STAT
2 protein dimers to interact and bind to adjacent DNA binding sites comprising measuring
3 the ability of a first preparation of a fragment of STAT protein dimer comprising the N-
4 terminal domain to bind to a second preparation of a fragment of said STAT protein
5 comprising the N-terminal domain in the presence and absence of a test compound,
6 wherein a test compound which increases the ability of the first preparation to bind to the
7 second preparation is identified as a drug that enhances the interaction between adjacent
8 activated STAT dimers, and a test compound which decreases the ability of the first
9 preparation to bind to the second preparation is identified as a drug that inhibits the
10 interaction between adjacent activated STAT dimers.

1 52. The method of Claim 51 wherein either said first preparation or said second
2 preparation is labeled.

1 53. The method of Claim 51 wherein said first preparation and said second preparation
2 are labeled.

1 54. The method of claim 51 wherein said first preparation is bound to a solid support.

1 55. The method of Claim 54 wherein said second preparation is labeled.

Add G-3 Add B-3